On-farm hatching and contact with adult hen post hatch induce sex-dependent effects on performance and welfare in broiler chickens

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Abstract

To improve the early perinatal conditions of broiler chicks, alternative hatching systems have been developed. On-farm hatching (OFH) with an enriched microbial and stimulating environment by the presence of an adult hen is a promising solution. Day-old certified JA 757 chicks were allotted within different hatching and rearing conditions: OFH, conventional hatchery (CH), CH and post-hatching treatment with antibiotics (CH + AB), as well as both hatching systems with an adult hen at hatching

(OFH + H, CH + H). On day (D) 27, chickens were challenged by combining transport in boxes in a new room at a lower temperature and fasting for 4 h. On their return to the original room, the chicken density was increased, and birds were orally vaccinated with the Gumboro vaccine. The impacts of these conditions on hatchability, chick quality score, performance, health and robustness were determined. The OFH chick body weights (BWs) were significantly greater than those of CH chicks at hatching. Whereas there was no effect of hatching conditions, the presence of hens, categorised according to their behaviour, decreased the hatchability rate, the quality score of OFH chicks and increased mortality at hatching. Treatment of CH chicks with antibiotics temporarily decreased chicken BW at D19, but the feed conversion ratio (FCR) was not modified. At D19, OFH chicks had the best BW compared to the other groups, and the presence of hens at hatching harmed chicken BW regardless of the hatching condition and FCR. An interaction between the effect of hatching conditions and chicken sex was observed later in BW. In males, the OFH chickens were the heaviest compared to the other groups at D34 but not at D56. The presence of hens eventually negatively impacted CH chicken BW at D56. In females, there was no effect of hatching condition on the BWs at D34 and D56, and the presence of hens eventually had a positive impact on OFH chicken BW. There was no effect of hatching conditions on health parameters. In conclusion, the OFH system was a hatching system at least equivalent to the CH system, if not better in this study. The effects of the hen's presence at hatching and during the chick start-up phase on performance interacted with the hatching condition and the sex of the chickens. The health status and brooding behaviour of the hens are essential to ensure the health and welfare of the chicks.

Introduction

In poultry, the perinatal period is a stressful period for broiler chicks, which includes the hatching phase and major physiological changes to adapt to new food resources and environments. In hatcheries, chicks hatch at between 19 and 21 days of incubation. They often stay more than 12 hours in the hatcher, under optimal temperature, without light and usually without access to feed and water until placement in farm buildings. The fasting period of the chicks is further increased by the time needed for hatchery processing, transportation duration and unloading at the farm, which might last up to the first 72 h after hatching. Even though chicks can use energy reserves from their yolk sac (van der Wagt et al., 2020), these conditions induce immediate and long-lasting metabolic changes (Beauclercq et al., 2019; Foury et al., 2020), behavioural impacts by increasing fear responses (Jessen et al., 2021) and consequences on chicken development, performance and welfare (de Jong et al., 2017).

To improve the early perinatal conditions of chicks, alternative hatching systems have been developed. On-farm hatching provides the chicks with immediate access to feed and water according to their needs and avoids the exposure to stressors encountered in conventional hatcheries (van de Ven et al., 2009). Eggs incubated for 18 days are transported to the farm and placed either in trays or in the litter where they hatch. The effects of these on-farm hatching systems on broiler health, welfare and performance were recently studied under commercial or more controlled conditions and had shown effects that are not always beneficial. Total mortality and footpad dermatitis in on-farm hatched (OFH) chicks were lower compared to conventionally hatched (CH) fast-

71 growing broiler chickens (de Jong et al., 2019; 2020; Giersberg et al., 2021; Jessen et
72 al., 2021). However, day-old chick quality was worse and breast myopathy prevalence
73 was higher for OFH than CH chickens (de Jong et al., 2019; Souza da Silva et al.,
74 2021).
75 Chicken activity and general behaviour were little affected by the hatching system, with
76 fast-growing OFH chickens being more fearful and less active (Giersberg et al., 2020).
77 Slower-growing broiler chickens hatched in organic farms tended to express less
78 general fearfulness than CH chickens (Jessen et al., 2021). A positive effect on growth
79 performance was observed during the first week of life until 21 days in OFH and CH
80 fed at the hatchery compared to CH chickens (de Jong et al., 2020), and longer when
81 parent flocks were young (Souza da Silva et al., 2021).
82 Maintaining optimal health, welfare and performance of chickens is highly dependent
83 on the gut physiology in interaction with the microbiota and mucosal immune system
84 (Fortun-Lamothe et al., 2023). Antibiotics have been largely used in poultry production
85 to improve performance by acting on the gut barrier function (Broom, 2018). However,
86 growing concerns about the increase of antimicrobial resistance in farm animals led to
87 changes in EU and national legislation governing the use of antibiotics as growth
88 promoters in poultry feed, which resulted in their suppression in 2006 (Council
89 Directive 96/22/EC; Axis 2 and measure 19 of the EcoAntibio 2017 plan). This made it
90 possible to become aware of the crucial role of the gut barrier and of the quality of the
91 microbiota implanted in the chick's gut at hatching on the physiological and immune
92 development, its robustness in the face of the hazards encountered during the chicks'
93 lives, and consequently on performance.

Greater attention to the environment during the chick postnatal period, especially the microbial environment, is key to optimising the gut barrier function and more broadly the health and welfare of the chickens and their performance. Naturally, chicks hatch in contact with an adult hen who is a donor of microbiota and a model of learning and maternal care (Edgar et al., 2016). Early implantation of adult microbiota into the chick digestive system accelerates the maturation of the microbiota and immune system (Volf et al., 2016; Broom & Kogut, 2018; Meijerink et al., 2020). In addition, chicks reared in the presence of their mothers are less fearful than those raised without their mothers and develop more behavioural synchrony (Perré et al., 2002), even though hen genetics has a strong effect on chick behaviour, with commercial lines being less maternal (Hewlett et al., 2019). The combination of a new hatching system like OFH with an enriched microbiota and stimulating environment from the presence of an adult hen is a possible solution for chick conditions to be improved and could contribute to poultry health and welfare and product quality.

In this study, we analysed the benefits/risks of hatching systems (OFH and CH), with an adult hen (OFH + H, CH + H) or not, and the combination of CH and post-hatching treatment with antibiotic growth promoter (CH + AB) on hatchability on chick quality score, performance, health and robustness.

Material and methods

All experimental procedures were approved by the Ethics Committee COMETHEA POITOU-CHARENTES n°84 (APAFIS#24474-2020021816237418 v3) and carried out following current European legislation (EU Directive 2010/63/EU). All steps of hatching,

117 experimentation and rearing were done at the experimental unit (EASM, Poultry
118 alternative breeding facility, INRAE, 17700 Surgères, France, DOI:
119 10.15454/1.5572418326133655E12).

120 **Experimental design**

121 Seven hundred twenty-day-old, certified JA 757 chicks, among which 432 were from a
122 conventional hatchery (CH) and 288 were hatched on-farm (OFH), were allocated into
123 five groups: CH, CH + antibiotics treatment (CH + AB), CH + hen (CH + H), OFH, OFH
124 + hen (OFH + H). Each group was randomly placed in the room, repeated in eight pens
125 (18 chicks/pen, 3 m^2). Antibiotic treatment was only applied in chick drinking water for
126 the CH + AB group: ADJUSOL® TMP SULF Liquid (25 mg/kg sulfadiazine and 5 mg/kg
127 trimethoprim, VIRBAC, CARROS, France) for 5 days (D2–D6) and SURAMOX 50 (400
128 mg/10 kg, i.e. 20 mg/kg amoxicillin, VIRBAC) for 5 days (D19–D23). Sex was
129 determined on tagged chickens on D19, and the number of chickens was adjusted to
130 a maximum of 16 per pen, keeping a balanced ratio between males and females. On
131 D27, chickens were challenged by combining transport in boxes to a new room at a
132 lower temperature (15 °C instead of 25 °C) and 4 h of feed deprivation. On their return
133 to the original room, the pen size was reduced from 3 m^2 to 1.5 m^2 to increase chicken
134 density, and birds were orally vaccinated with the live Gumboro vaccine in drinking
135 water (HIPRAGUMBORO® - G97, HIPRA FRANCE, Saint-Herblain, France). Chickens
136 had ad libitum access to feed without anticoccidial drugs. They were fed with a
137 standard starter diet (raw energy = 4462 kcal/kg, crude protein = 23.91%) until D19,
138 then a grower diet from D20 to D34 (4527 kcal/kg, crude protein = 20.51%) and a

finisher diet from D35 to D56 (4600 kcal/kg, crude protein = 19.98%). Faeces were collected from litter on D14 and D54 for parasite analyses.

Hatching and husbandry

Hatching conditions

Certified JA 757 18-day embryonated eggs (Galina Vendée, Essarts-en-Bocage, France) were either placed at 37.6°C with 75% relative humidity and no light in a conventional hatchery (CH) or laid directly in the litter of the pens under infrared heat lamps to allow on-farm hatching (OFH). The average temperature of the eggs in the litter was 37.9°C and under 20 h light per day until OFH chick hatching. The ambient room temperature was maintained at 25 °C with a fan heater. Day-old CH chicks were transported for one hour in a transport van before placement in pens to simulate conventional hatchery processing, which has been described to have long-term deleterious effects on fear response when combined with delayed nutrition (Hollemans et al., 2018). Both CH and OFH chicks were placed under heat lamps. In pens hosting hens, 18-day embryonated eggs or chicks were placed in a gridded space under the heat lamp. Temperatures under heat lamps were decreased from 35–38 °C to 31–32 °C from D0 to D3, then 29–30 °C from D4 to D6 and 26–27 °C from D7 to D13. The light cycle was 20 h light at the CH chick placement or until hatching time for OFH chick (D0), 13 h light on D1 (increased dark time to promote maternal behaviour of hens), 18 h on D2 and 16 h on D3 and during the rearing period with minimum 20 lux on 80% of the lighted surface.

Chicks had ad libitum access to water and food; a wire mesh platform and a perch were used for environmental enrichment.

Contact with hens

Sixteen Lohmann Brown hens, acting as natural sources of gut microbiota and adult presence, were obtained from a local commercial egg-laying hen farm (La cabane à Chiron, Benet, France). The hens were aged 31 weeks, vaccinated against Marek Disease Virus (MDV), Infectious Bursite Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV) infections, and were sanitary controlled and declared free of *Mycoplasma gallisepticum*, *Mycoplasma synoviae, Chlamydia psittaci* and *Salmonella pullorum gallinarum.* Only *Ascaris* and *Heterakis* parasites were detected in hen faeces, and they were at a very low level.

Each hen was placed separately in a wire-latticed pen (3 m^2) in the experimental pens described above with a nest box, perch, feed and water ad libitum. Hens were accustomed to their new environment for 12 days, fed with a standard rearing diet for laying hens (30099G25, Arrivé Nutrition Animale, Saint-Fulgent, France) and allowed to deposit faecal and caecal microbiota on litter. The room temperature was 25 °C and the artificial photoperiod was 16 h L:8 h D before egg deposition, 20 h L:4 h D during hatching and the same programme as the chicks afterwards. Two days before chick arrival or egg hatching, a wire-latticed space for chicks was placed in their pen. Eight hens were used for 8 groups of 18 OFH chicks, and eight hens were used for 8 groups of 18 CH chicks. On D0, day-old CH chicks were placed under the pen's wire-latticed space, and OFH chicks were already under this space. Chicks and hens were in visual and auditory contact for a few hours. Then hens were deprived of feed and water from the morning. When lights were switched off, the hens were shut up in their nest boxes, and chicks were placed under each hen as gently as possible for 11 h without any feed and water. Chicks and hens were put physically together in a closed nest for the night

186 to promote maternal behaviour and the acceptance of chicks (Richard-Yris &
187 Leboucher, 1987). The following morning, one hour before the lights were switched on,
188 the nest-box doors were taken away to allow free access to the whole pen. Free in-
189 access feed and water were placed under wire-latticed space for chicks and in raised
190 troughs for hens, not accessible for chicks. Hens were present with chicks for two
191 weeks, the critical period for chick start, and removed on D15. Weight and clinical
192 examinations of the hens were recorded the day before they were installed in the pens
193 and, on D15, when they were removed.

194 **Behavioural observations**

195 The scan sampling method was used to follow the behaviour of hens and chicks on
196 days 2, 5, 6, 7, 8, 9, 12, 13 and 14 with the following repertoire: resting (the hen is lying
197 or standing still, eyes closed and without chicks), maintenance (preening, scratching,
198 stretching), feeding behaviour (the hen is eating or drinking), locomotion, exploration
199 (the hen is scratching or pecking at the ground or the environment), observation (the
200 hen is observing the environment with neck movements), maternal behaviour (the hen
201 is making food offering to the chicks, the hen is expressing maternal calls, the hen is
202 brooding the chicks by lying down and spreading her wings), fear behaviour (the hen
203 is flying or running from the experimenter, freezing, alert), agonistic behaviour (the hen
204 is chasing the chicks, the hen is pecking the chicks, others (punctual behaviours like
205 vocalisations).
206 To evaluate the proximity between chicks and hens, the experimenter also recorded
207 the localisation of four randomly tagged chicks and the hen within the pen. To that end,
208 the pen was virtually divided into four zones (Figure 1). The observations were

209 conducted between 10 AM and noon and between 3 and 5 PM by the same
210 experimenter. The experimenter walked slowly in front of each pen and recorded the
211 behaviour of the hen and the localisation of the four tagged chicks every eight minutes
212 (approximately), with a total of 10 scans per hen per day and 177 scans per hen for
213 the whole period of observation.
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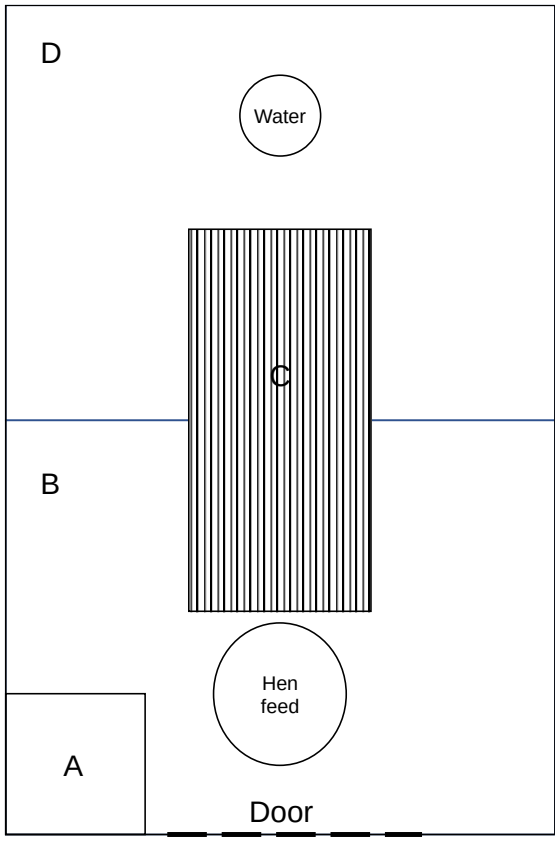

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217 Figure 1. Schematic representation of the pen (3m^2) with the zones used to locate the
218 four tagged chicks and the hen during behavioural observations; A: the nest, B and D:
219 two halves of the pen and C: the wire-latticed space for the chicks (101 × 50 cm).
220

Chick quality scores

Chick quality scores were determined at placement in the pen for CH chicks, corresponding to 21 days of incubation for OFH chicks, on 24 to 25 chicks from the three treatments: CH, OFH and OFH + H. They were macroscopically defined according to the grid of Tona (Tona et al., 2003) and modified by adding several other parameters issued from the CASDAR QUALICOUV project (Guinebretière et al., 2022). Briefly, the chicks were scored on a total score of 110, including scores of posture (on 5), down (on 5), legs (on 6), red dot on the beak (on 10), grouped into an "*appearance*" score (on 26); activity (on 6), eyes (on 16), leg joint inflammation (on 5) and leg dehydration (on 5) were grouped into a "*tiredness"* score (32), and finally, retracted yolk (on 12), navel (on 12), remaining membrane (on 12), and remaining yolk (on 16) were grouped in an "*abdomen"* score (on 52).

Health parameters

Droppings deposited on pen litter were collected on D14 and D54 and analysed for parasite detection (*Coccidia*, *Ascaris* and *Heterakis*). Five grams of droppings were homogenised in 70 mL of flotation solution (0.36% of sodium chloride). The mixture was then filtered and pressed through a tea strainer (small mesh) to extract as much of the liquid part as possible. A homogeneous sample was deposited into a McMaster cell counter, and after 5 min of rest, the oocysts and nematode eggs were counted, and their number was expressed per gram of droppings (OPG). Health disorders, mortality and causes of death were registered during the experiment.

Performance

Body weight (BW) was measured at D1, D19, D34 and D55. Feed consumption was measured in each pen for the periods between D1–D19, D19–D34 and D34–D55, and then used to calculate the feed conversion ratio (FCR) as the feed consumption-to-BW gain ratio per pen during both periods and the entire rearing period. At D56, 16 identified males per group were slaughtered, and *pectoralis major* and *pectoralis minor* (breast) muscles were weighed to calculate their yields relative to BW and ultimate pH. Ultimate pH was measured as the pectoralis major pH 24 hours after slaughter.

Statistical analyses

Hatching rates between hatchery and on-farm hatchings were compared using chi-squared tests. Chick quality parameters were analysed by a non-parametric Kruskal-Wallis test, considering the treatment (CH, CH + H, OFH and OFH + H), followed by Mann-Whitney post hoc tests. The normality of residual distribution was checked with the Shapiro-Wilk test for BW, feed intakes and FCR. A 2-way ANOVA was then carried out to test the hatching condition and the sex effect, as well as the two-by-two interactions on performance. When there was an interaction between variables, a Fisher (LSD) test was used to determine the statistical significance of the difference. Differences were considered significant when p-values < 0.05 and a tendency for $0.1 < p < 0.05$. Analyses were performed using XLSTAT software (version 2015, Addinsoft, Paris, France).

Behavioural data did not meet the assumption of normality and homogeneity of variances. Non-parametric Mann-Whitney U-tests were used on the mean percentage

of scans per behavioural category to compare the behaviour of hens in contact with CH chicks to the hens in contact with OFH chicks. To compare the proximity of CH and OFH chicks towards the hen, Mann-Whitney U tests were conducted on the mean number of tagged chicks located in the same area of the pen as the hen over the 177 scans recorded per hen.

Results

Behavioural observations

Because 3 hens (1 OFH + H and 2 CH + H) were very aggressive and injured their chicks, they were removed from the pens and the later behavioural analysis. There was no significant difference in the behaviour of the hens, regardless of the hatching condition of chicks, except for the frequency of the behaviour "*observe*"; OFH hens tended to observe their environment less than CH hens (Table 1).

Table 1. Behaviour of hens according to the chick hatching conditions

Hen behaviour	Hatching conditions		*P*-value
	CH	OFH	
Agonistic	2.54 ± 3.74	1.37 ± 0.72	0.550
Rest/Comfort	17.72 ± 7.16	31.16 ± 22.74	0.181
Fear	7.07 ± 3.39	4.92 ± 1.95	0.384
Feeding	18.10 ± 4.52	19.45 ± 11.56	0.731
Locomotion	6.78 ± 4.12	3.39 ± 2.95	0.146
Observation	17.53 ± 7.45	9.52 ± 4.76	0.045
Exploration	22.62 ± 7.62	19.77 ± 10.78	0.656
Maternal	1.32 ± 1.94	3.39 ± 7.98	0.732
Others	6.32 ± 2.31	7.02 ± 7.02	0.470

CH = conventional hatchery (n = 6), OFH = on-farm hatching (n = 7)
Behaviour observations (mean ± SD of scan percentage over 9 days)
P-value < 0.05 = significant difference between hatching conditions (Mann-Whitney U-test)

To characterise hens' behaviour towards the chicks, each hen was categorised according to the frequencies of agonistic or maternal behaviours (Table 2). We defined three categories: 1) maternal (M): the hens expressed only maternal behaviours towards the chicks; 2) tolerant (T): the hens expressed both maternal and agonistic behaviours towards the chicks or less than 2% of scans with maternal behaviour; 3) aggressive (A): the hens rejected the chicks and expressed only agonistic behaviour towards them. Two hens were defined as maternal, six were tolerant, and five were aggressive among the 13 hens analysed (Table 2).

Table 2. Classification of hen according to the frequencies of maternal or agonistic behaviours expressed towards chicks.

Hatching conditions	Hen behaviours		Category
	Agonistic	Maternal	
CH1	7.91 ± 0.27	0	A
CH2	0	0.57 ± 0.07	T
CH3	0.56 ± 0.07	0.56 ± 0.07	T
CH4	0	5.08 ± 0.22	M
CH5	0	1.69 ± 0.13	T
CH6	6.78 ± 0.25	0	A
OFH1	1.13 ± 0.11	0	A
OFH2	0	21.47 ± 0.41	M
OFH3	1.69 ± 0.13	0.56 ± 0.07	T
OFH4	1.69 ± 0.13	0.56 ± 0.07	T
OFH5	1.13 ± 0.11	1.13 ± 0.11	T
OFH6	1.69 ± 0.13	0	A
OHF7	2.26 ± 0.15	0	A

Thirteen hens were classified, three agressive hens (1 OFH+H and 2 CH+H) were removed from the analysis
CH = conventional hatchery; OFH = on-farm hatching
Behaviour observations (mean ± SD of scan percentages over 9 days)
A = Agressive (maternal behaviour = 0)
T = Tolerant (0 < maternal behaviour < 5)
M = Maternal (maternal behaviour > 5 and agonistic behaviour = 0)

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300 The mean number of chicks observed in the same area as the hen did not differ

301 significantly between CH and OFH chicks ($p > 0.05$). (Figure 2).

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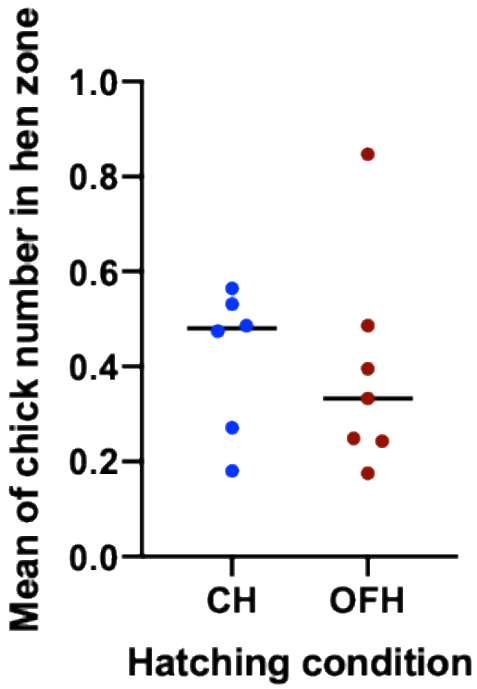

Figure 2. Proximity between chicks and hens according to hatching conditions; four chicks were observed per pen (n ≤ 8 scans per day) per hatching condition (conventional hatchery, CH or on-farm hatching, OFH)

Hatchability and chick quality

Hatchability

For conventional hatchers, 97.7% of CH fertile eggs hatched at E21 and 97.2% ± 4.2% of OFH fertile eggs hatched at E21 in pens. The presence of hens had a significant impact on the OFH condition ($p = 0.034$). In the presence of hens, 86.8% ± 11.9% of OFH + H chicks hatched at E21 (Figure 3). Unhatched eggs were mainly found in the pens with aggressive hens (9/11) or in the OFH pens next to those with aggressive hens (4/4). No mortality of CH chicks or OFH chicks was observed at hatching, whereas 5.6% ± 5.9% OFH + H chicks died or were removed at hatching (n = 10); due

316 to three hens' aggressiveness or another reason. Only 3.6% (2/56) of chicks had
317 residual yolk sacs at the age of 20 days (one CH and one CH + AB) and no yolk residue
318 was found at 56 days.
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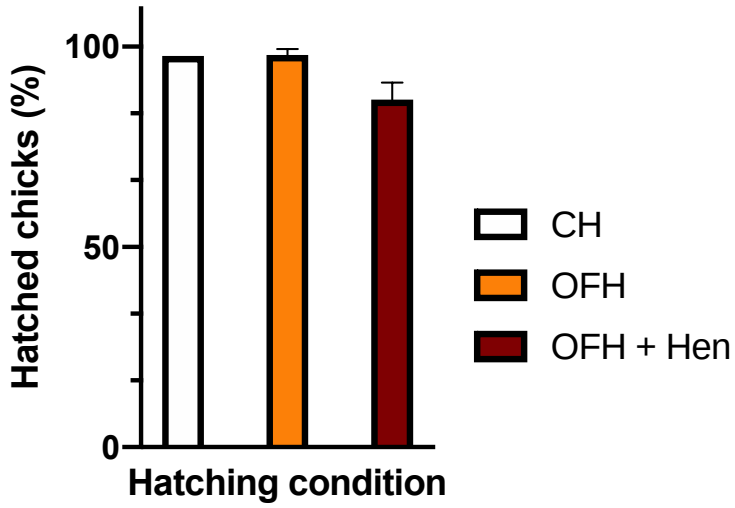

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322 Figure 3. Number of live hatched chicks according to hatching conditions; conventional
323 hatchery (CH) condition performed in one hatchery (one value); on-farm hatching
324 (OFH) and on-farm hatching with hen (OFH + Hen) conditions were repeated in eight
325 pens each, each pen contained 18 embryonated eggs or chicks; values are expressed
326 as means ± standard error

327 *Quality scores of chicks*
328 Whereas no difference was shown due to the hatching conditions ($p > 0.05$) on the
329 total quality scores, with good scores in the three groups considered (OFH: 96.2 ± 1.5,
330 CH: 97.3 ± 1.5; CH+H: 95.1 ± 1.7), when considering the subtotal scores linked to the
331 appearance, the tiredness or the abdomens of the chicks, it appeared that the subtotal

of the appearance score changed depending on the treatment (Figure 4), with the two other subtotals not being significantly changed ($p > 0.05$, data not shown). Indeed, whereas the subtotal score for appearance was not different between CH chicks or OFH chicks, it was deteriorated by the presence of the hen within the hatching pen in OFH chicks ($p = 0.01$).

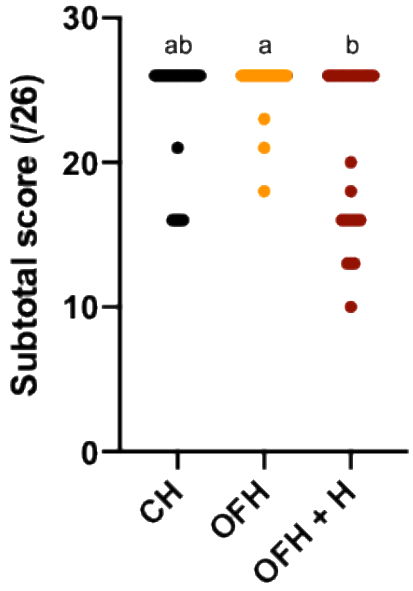

Figure 4. Chick appearance subtotal score at the placement in the pen according to hatching conditions; appearance scores noted on 26 included scores of posture (on 5), down (on 5), legs (on 6), and a red dot on the beak (on 10); n = 24 to 25 chicks/hatching condition; conventional hatchery (CH), on-farm hatching (OFH), OFH + hen (OFH + H)

Performance

Hatching conditions significantly influenced chick BW from hatching to slaughter age. The OFH chick BW was significantly greater than that of all CH chicks at hatching,

whether hens were present or not ($p \leq 0.002$, Figure 5). Independently of the treatment, a sex effect was observed from D19 onwards; male chicken BWs were greater than those of females (males: 503 ± 46g, females: 469 ± 37g, $p = 0.0001$). Treatment of CH chicks with antibiotics temporarily decreased chicken BW at D19 ($p = 0.035$) (Figure 5) due to a decrease in weight gain (CH: 497 ± 38g, CH + AB: 486 ± 37g, $p = 0.0001$) compared to CH chickens, while feed intake (data not shown) and FCR were not different (Table 3). At D19, OFH chickens had the best BW compared to all other groups of chicks ($p \leq 0.0003$) (Figure 5). At this time, the presence of hens at hatching had a remnant negative impact on chicken BW regardless of the hatching condition ($p < 0.0001$), as well as on FCR (Table 3). Both the feed intake per chicken (CH: 624 ± 12g^{a}, CH + AB: 600 ± 27g^{ab}, CH + H: 603 ± 25g^{bc}, OFH: 652 ± 33^{a}, OFH + H: 615 ± 34^{c}, $p = 0.001$) and the weight gained per chicken (CH: 455 ± 37g^{b}, CH + AB: 445 ± 37g^{c}, CH + H: 421 ± 40g^{d}, OFH: 471 ± 42g^{a}, OFH + H: 425 ± 47g^{d}, $p = 0.0001$) decreased compared to the other groups, and the FCR increased (Table 3). An interaction between the effect of the hatching condition and chicken sex on BW was observed later at D34 ($p = 0.012$) and D56 ($p = 0.022$) on BW, even though the FCR was not affected (Table 3). At D34, a week after the challenge, the OFH male chickens were the heaviest compared to the other groups ($p \leq 0.033$) and the presence of hens at hatching harmed chicken BW ($p \leq 0.0004$), regardless of the hatching condition (Figure 6A). In females, there was no effect of hatching condition or presence of hens on the BW at D34 (Figure 6A). At slaughter age (D56), there was no effect of hatching condition on the male chicken BW, but the presence of hens at hatching harmed CH chicken BW ($p = 0.0008$) (Figure 6B). There was a pen effect in CH + H ($p = 0.016$) and OFH + H chickens ($p = 0.001$), the pen with the lightest CH + H males was in the

371 presence of an aggressive hen, and the heaviest OFH + H males were in a pen in the
372 presence of a tolerant hen, but all combinations were observed (Figure 7). In females,
373 there was no effect of the hatching condition on the BW. The presence of hens at
374 hatching had a positive impact on OFH female chickens compared to CH female
375 chicken BW ($p = 0.0096$), with the OFH + H chickens being the heaviest compared to
376 the other CH female conditions (Figure 6B). There was no significant pen effect
377 between CH + H and OFH + H female chickens ($p = 0.447$).
378

Table 3. Performance according to the hatching conditions of chicks

Day ranges	Feed conversion ratio (g/g)					
	CH	CH + AB	CH + H	OFH	OFH + H	
D1–D19	1.370 ± 0.024c	1.350 ± 0.066c	1.416 ± 0.049ab	1.388 ± 0.022bc	1.447 ± 0.035a	0.001
D20–D34	1.807 ± 0.030	1.773 ± 0.042	1.769 ± 0.039	1.795 ± 0.035	1.787 ± 0.057	0.355
D35–D55	2.194 ± 0.091	2.213 ± 0.055	2.188 ± 0.054	2.201 ± 0.049	2.141 ± 0.038	0.173
D1–D55	1.904 ± 0.036	1.902 ± 0.025	1.913 ± 0.040	1.910 ± 0.022	1.912 ± 0.015	0.924

Hatching conditions: conventional hatchery (CH), CH + antibiotics treatment (CH + AB), CH + hen (CH + H), on-farm hatching (OFH), OFH + hen (OFH + H)
Values are expressed as mean ± standard error
a,b,c, Different letters correspond to significant differences between treatment groups

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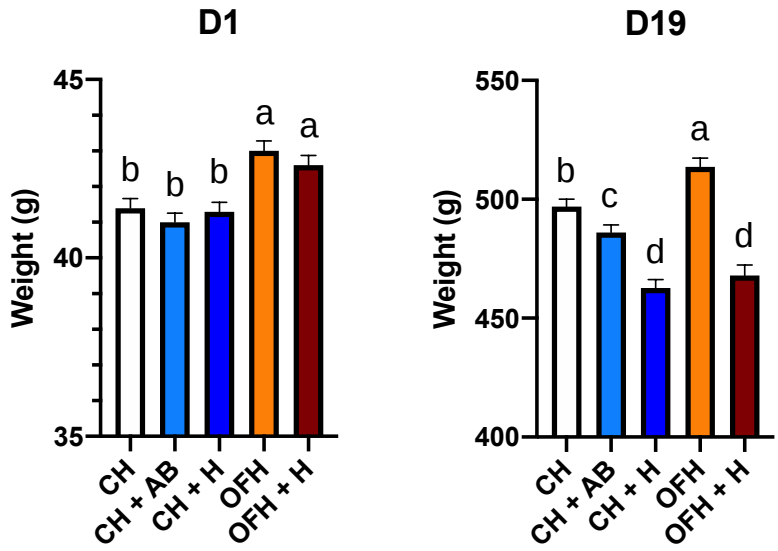
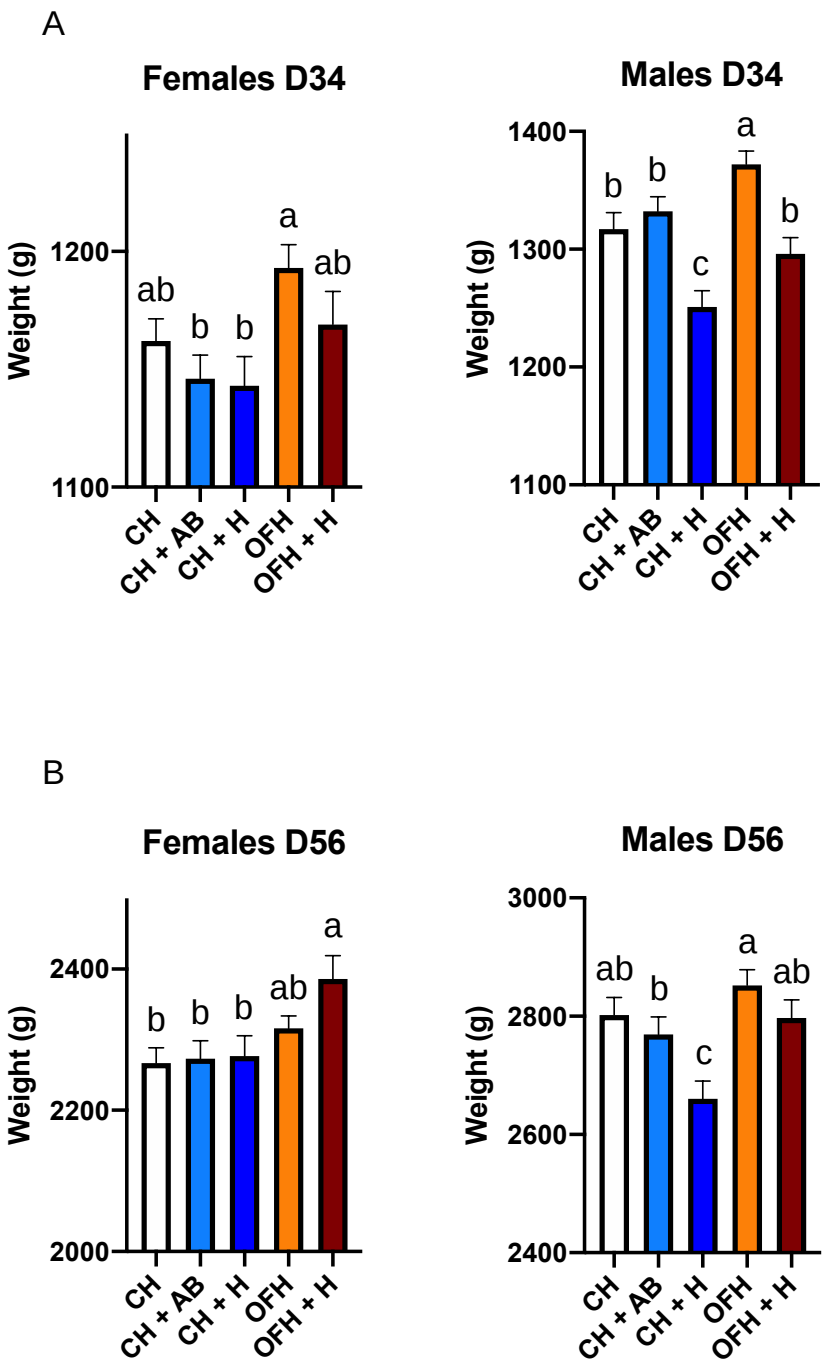


Figure 5. Body weight at D1 and D19 and according to the hatching conditions: conventional hatchery (CH), CH + antibiotics treatment (CH + AB), CH + hen (CH + H), on-farm hatching (OFH), OFH + hen (OFH + H); values are expressed as means ± standard error; different letters correspond to significant differences between treatment groups



386

387 Figure 6. Weight at D34 (A) and D56 (B) of male and female chickens according to the
388 hatching conditions: conventional hatchery (CH), CH + antibiotics treatment (CH + AB),
389 CH + hen (CH + H), on-farm hatching (OFH), OFH + hen (OFH + H); values are
390 expressed as mean ± standard error: different letters correspond to significant
391 differences between treatment groups

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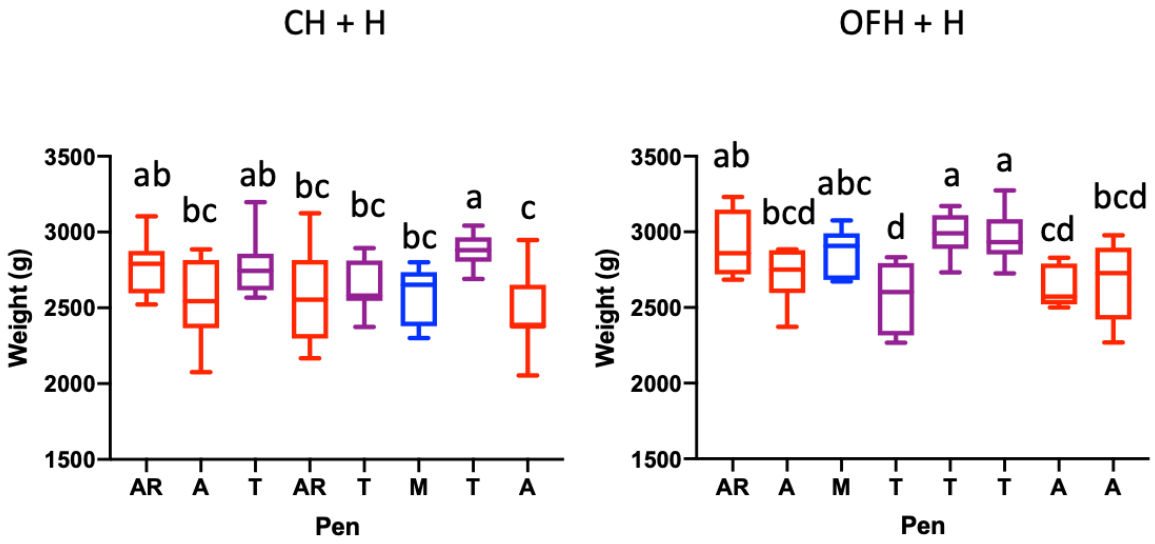

393

394 Figure 7. Body weight at D56 of male chickens according to the behaviour of the hen
395 present at the starting period, M: maternal, T: tolerant, A: aggressive, AR: aggressive
396 and removed from the pen; CH + H: chicks hatched in the hatchery and in the presence
397 of hens; OFH + H: chicks hatched on-farm in the presence of hens; median ± SD (n ≤
398 9).

399

400 Breast weight was not affected by the hatching conditions (6.99 ± 0.06, p = 0.357) and
401 ultimate pH was not modified either (5.7 ± 0.1, p = 0.951).

402 **Health and robustness**

403 *Coccidia* was detected in variable amounts in the droppings of all the pens at D54
404 (200–85500 OPG) without any significant effect of the hatching conditions in the
405 presence of hen or not (p = 0.606). No clinical signs were observed during the
406 experiment. In all hatching conditions combined, the viability rate of the chickens was
407 95.3%. The mortality rate during the whole experiment was 3.19% (23/720). Seventeen

chicks died during the first week of life, 11 OFH + H and 5 CH + H in the presence of hens and one OFH chick for an unknown reason. Six CH chickens died during the rest of the experiment, five of which were due to heart problems (2 CH, 1 CH + AB, 2 CH + H) and one to unknown causes (CH + H). Eleven chicks were additionally eliminated after hatching in pens in the presence of hens (4 at D1, 4 at D2, and 1 at D4) and two later (D33 and D55) for morphological reasons.

Discussion

New hatching systems are being developed in Europe, and the enrichment of the rearing environment is also in full development, notably by optimising the microbial environment of the chicks to limit the use of antibiotics. In this study, we analysed the benefits/risks of hatching systems (OFH and CH, treated with antibiotics or not) and of the presence of an adult hen or not on hatchability, chick quality score, performance, health and robustness.

Hatching conditions

The BW of OFH-certified JA757 chicks was significantly greater than that of CH chicks at hatching, even though the hatchability rate and the quality score of chicks were comparable between the two conditions, and no mortality was reported. These results agree with other studies on OFH performed in slow (Jessen et al., 2021) and fast-growing broilers (de Jong et al., 2020; Souza da Silva et al., 2021) for the BW but not for other parameters that were reported higher for the hatchability, lower for the quality score of chicks and lower for the mortality. However, whereas there was no effect of hatching conditions, the presence of hens, categorised according to their behaviour,

decreased the hatchability rate, the appearance quality score of OFH chicks and increased mortality at hatching. These degraded indicators could be since in our experimental design, very few hens expressed a clear maternal behaviour towards the chicks (n = 2/16), and some even showed agonistic behaviour. This may be explained by the genetic line of hens used (Lohmann Brown), which is highly selected for laying and counter-selected for brooding. However, this genetic line was chosen because the studied practice could favour the possibility to use culled hens in breeding, and because of their rather tolerant social behaviour, their brooding behaviour could be optimised. Improvements could be obtained by carrying it out in a season with days with greater light amplitudes (spring) to facilitate brooding behaviour, which was not the case in this study (winter), and by selecting hens with brooding behaviour to facilitate maternal behaviour (Shimmura et al., 2010). In addition, in our experimental design, the chicks had to feed under the wire-lattice space, which was not accessible to the hen. As they obtained both food and warmth under this space, the hens probably did not have enough tactile stimulation from the chicks to fully express their maternal behaviour with no agonistic behaviour. Indeed, in addition to the physiological state, tactile stimulations from chicks play an important role in the expression and maintenance of maternal behaviour in hens (Richard-Yris & Leboucher, 1987).

Starting period

Hatching conditions and the presence of hens for 15 days after placement significantly influenced chick performance during the starting period. At D19, OFH chicks had the best BW compared to the other groups. and the presence of hens at hatching harmed chicken BW regardless of the hatching condition and on FCR. No significant differences were observed in the behaviour of hens present with OFH and CH chicks,

except for OFH hens, which were found to observe their environment less than CH hens. With our small sample size, this result could be explained by the behaviour of one OFH hen, which spent much of the time resting. The CH and OFH chicks did not differ in their proximity towards the hen. The mean number of chicks observed in the same area as the hen was very low (less than 1 chick), indicating that they were rarely in contact with the hen. However, chick performance was affected by the presence of the hens, including lower feed intake and consequently lower weight gain and higher FCR. This could be explained by the agonistic behaviour of some hens towards chicks, the attempt of the hens to eat the chick feed and the stress that this may have caused the chicks.

Treatment of CH chicks with antibiotics, assessed as growth promoters, temporarily decreased chicken BW at D19, but FCR was not modified. This effect was not observed later, but growth promotion was not observed in CH chicks treated with antibiotics. This result is not in agreement with the use of antibiotics as growth promoters in farm animals, but the relative lack of published data on chicken performance limits knowledge of the actual effects of antibiotics on animal performance (Kumar et al., 2018; Broom, 2018). Their effects also result from their interaction with the microbiota and the variables chosen in the experimental studies. The effects observed in farms are dependent on the sanitary conditions present, which are different from the much more controlled sanitary conditions in the experimental studies and may contribute to different effects of treatment with antibiotics.

Growth period

An interaction between the effect of hatching conditions and chicken sex was observed on BW after the challenge on D27. In males, the OFH chicken group was the heaviest

compared to the other groups at D34 but not at D56. These results are consistent with a previous study that observed the beneficial effects of OFH on BW only until D21 (de Jong et al., 2020), and not until slaughter time, as reported in various studies when post-hatching feed deprivation time was at least 36 h (de Jong et al., 2017). Alternatively, this may also be a result of the response to the challenge experienced by the chickens at D27, including transport, exposure to low temperature, transient feed deprivation, vaccination and a change to a higher rearing density, but the fact is that there is no ultimate positive impact of OFH on BW at slaughter time. Moreover, in our conditions, the presence of hens eventually negatively impacted male chicken BW, but only for CH chickens at D56. In females, there was no effect of hatching conditions on the BW at D34 and D56, and the presence of hens eventually had a positive impact on OFH female chicken BW. These results were unexpected, but it is known that early stress induces sex-specific, immediate and life-long effects on the stress response, behaviour, sex hormones, and hypothalamic and blood gene expression in chickens (Madison et al., 2008; Elfwing et al., 2015; Foury et al., 2020), with the males being more reactive than the females. The results observed in this study raise questions about the consequences of hatching conditions in the presence of a hen according to the sex of the chicks. It appeared that male OFH chicks developed more fear and stress responses than females when placed in the presence of a hen that was not their mother, and this had effects on their growth until slaughter age. For male OFH chicks, in which the effect of hen presence on their growth was only observed during the growth phase, there was possibly communication between hens and embryonated eggs before hatching and with chicks at hatching that may have increased their fear and stress responses and therefore harmed their growth. This could even have had negative consequences on hatchability and mortality rates, but the sex of the chicks

was not recorded at that time. The presence of hens with the female OFH chicks did not affect their performance and even had a beneficial effect on their growth at slaughter age. These differences observed between treatments and chick sexes for performance are not likely explained by a difference in proximity between hens and chicks, which was low in this experiment.

Health and Robustness

There were no effects of hatching conditions on health parameters (parasitic load, clinical signs, rate of mortality), even after exposure of chickens during their growth phase to an environmental and vaccine challenge. However, this challenge could have accentuated the differences in the effects of hatching conditions on performance parameters between males and females, but we did not perform the unchallenged rearing conditions to assert this. The implantation of adult microbiota into the chick digestive system by the presence of hens should be nevertheless beneficial for the maturation of the chick microbiota and gut immune system and still needs to be assessed.

Altogether, on-farm hatching of certified broilers was a hatching system at least equivalent to the hatchery hatching system, if not better, in this study. The effects of the hens' presence at hatching and during the chick start-up phase on performance interacted with the hatching condition and the sex of the chickens. In this case, the health status and brooding behaviour of the hens are essential to ensure the health and welfare of the chicks. These practices offer possible evolutions of the rearing conditions to continue to decrease the use of antibiotics.

Ethics approval

All experimental procedures were approved by the Ethics Committee COMETHEA POITOU-CHARENTES n°84 (APAFIS#24474-2020021816237418 v3) and carried out following current European legislation (EU Directive 2010/63/EU).

Author contributions

LAG, AB, CS, KG and AC designed the study with the help of CB. LAG, CB and AC performed the experiment with the technical help of SC for the organisation of the experiment and AH for parasitic analyses. CB and LR collected the performance and health parameters. LAG analysed data with the help of AB and CB for the behaviour data. LAG, AB and CB wrote the paper with the help of KG and AC. All the authors reviewed and approved the manuscript.

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Acknowledgements

We are grateful to all the members of the RIMEL network whose shared thinking made the design of this study possible. We thank the staff of the poultry alternative breeding experimental unit (EASM, INRAE, 17700 Surgères, France, DOI: 10.15454/1.5572418326133655E12) for the development of the experimental set-up and the conduct of the experimentation. We are very grateful to the staff of the MOQA team (INRAE, 37380 Nouzilly, France) for their help during the experimentation. The manuscript has been professionally proofread.

Funding

This research was supported by a grant from INRAE, Department of Animal Physiology and Livestock Systems for the RIMEL network.

Data and model availability statement

The datasets used during the current study are available on line:
https://doi.org/10.57745/6INVYL

References

Beauclercq S, Lefèvre A, Montigny F, Collin A, Tesseraud S, Leterrier C, Emond P, Guilloteau LA (2019) A multiplatform metabolomic approach to characterize fecal signatures of negative postnatal events in chicks: a pilot study. J Anim Sci Biotechnol, 10, 21. https://doi.org/10.1186/s40104-019-0335-8

567 Broom LJ (2018) Gut barrier function: Effects of (antibiotic) growth promoters on key
568 barrier components and associations with growth performance. Poult Sci, 97,
569 1572-1578. https://doi.org/10.3382/ps/pey021
570 Broom LJ, Kogut MH (2018) The role of the gut microbiome in shaping the immune
571 system of chickens. Vet Immunol Immunopathol, 204, 44-51.
572 https://doi.org/10.1016/j.vetimm.2018.10.002
573 de Jong IC, van Riel J, Bracke MBM, van den Brand H (2017) A 'meta-analysis' of
574 effects of post-hatch food and water deprivation on development, performance
575 and welfare of chickens. PLoS One, 12, e0189350.
576 https://doi.org/10.1371/journal.pone.0189350
577 de Jong IC, Gunnink H, van Hattum T, van Riel JW, Raaijmakers MMP, Zoet ES, van
578 den Brand H (2019) Comparison of performance, health and welfare aspects
579 between commercially housed hatchery-hatched and on-farm hatched broiler
580 flocks. Animal, 13, 1269-1277. https://doi.org/10.1017/S1751731118002872
581 de Jong IC, van Hattum T, van Riel JW, De Baere K, Kempen I, Cardinaels S, Gunnink
582 H (2020) Effects of on-farm and traditional hatching on welfare, health, and
583 performance of broiler chickens. Poult Sci, 99, 4662-4671.
584 https://doi.org/10.1016/j.psj.2020.06.052.
585 Edgar J, Held S, Jones C, Troisi C (2016) Influences of maternal care on chicken
586 welfare. Animals (Basel), 6, 2. https://doi.org/10.3390/ani6010002
587 Elfwing M, Nätt D, Goerlich-Jansson VC, Persson M, Hjelm J, Jensen P (2015) Early
588 stress causes sex-specific, life-long changes in behaviour, levels of gonadal
589 hormones, and gene expression in chickens. PLoS One, 10, e0125808.
590 https://doi.org/10.1371/journal.pone.0125808

591 Fortun-Lamothe L, Collin A, Combes S, Ferchaud S, Germain K, Guilloteau L, Gunia
592 M, Lefloc'h N, Manoli C, Montagne L, Savietto D (2023) Principes, cadre
593 d'analyse et leviers d'action à l'échelle de l'élevage pour une gestion intégrée de
594 la santé chez les animaux monogastriques. INRAE Prod Anim, 35, 307–326.
595 https://doi.org/10.20870/productions-animales.2022.35.4.7225
596 Foury A, Collin A, Helbling JC, Leterrier C, Moisan MP, Guilloteau LA (2020)
597 Spontaneous intake of essential oils after a negative postnatal experience has
598 long-term effects on blood transcriptome in chickens. Sci Rep, 10, 20702.
599 https://doi.org/10.1038/s41598-020-77732-5
600 Giersberg M F, Poolen I, de Baere K, Gunnink H, van Hattum T, van Riel J W, de Jong
601 IC (2020). Comparative assessment of general behaviour and fear-related
602 responses in hatchery-hatched and on-farm hatched broiler chickens. Appl Anim
603 Behav Sci, 232, 105100. https://doi.org/10.1016/j.applanim.2020.105100
604 Giersberg MF, Molenaar R, de Jong IC, Souza da Silva C, van den Brand H, Kemp B,
605 Rodenburg TB (2020) Effects of hatching system on the welfare of broiler
606 chickens in early and later life. Poult Sci, 100, 100946.
607 https://doi.org/10.1016/j.psj.2020.12.043
608 Guinebretière M, Puterflam J, Keïta A, Réhault-Godbert S, Thomas R, Chartrin P,
609 Cailleau-Audouin E, Coudert E, Collin A (2022) Storage temperature or thermal
610 treatments during long egg storage duration influences hatching performance
611 and chick quality. Front Physiol, 13, 852733.
612 https://doi.org/10.3389/fphys.2022.852733
613 Hewlett SE, Nordquist RE (2019) Effects of maternal care during rearing in White
614 Leghorn and Brown Nick Layer Hens on cognition, sociality and fear. Animals
615 (Basel), 9, 454. https://doi.org/10.3390/ani9070454

616 Hollemans MS, de Vries S, Lammers A, Clouard C (2018) Effects of early nutrition and
617 transport of 1-day-old chickens on production performance and fear response.
618 Poult Sci, 97, 2534-2542. https://doi.org/10.3382/ps/pey106
619 Jessen CT, Foldager L, Riber AB (2021) Effects of hatching on-farm on performance
620 and welfare of organic broilers. Poult Sci, 100, 101292.
621 https://doi.org/10.1016/j.psj.2021.101292
622 Kumar S, Chen C, Indugu N, Werlang GO, Singh M, Kim WK, Thippareddi H (2018)
623 Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics,
624 immunity, growth performance and prevalence of foodborne pathogens. PLoS
625 One, 13, e0192450. https://doi.org/10.1371/journal.pone.0192450
626 Madison FN, Jurkevich A, Kuenzel WJ (2008) Sex differences in plasma corticosterone
627 release in undisturbed chickens (Gallus gallus) in response to arginine vasotocin
628 and corticotropin releasing hormone. Gen Comp Endocrinol, 155, 566-573.
629 https://doi.org/10.1016/j.ygcen.2007.08.014
630 Meijerink N, Kers JG, Velkers FC, van Haarlem DA, Lamot DM, de Oliveira JE, Smidt
631 H, Stegeman JA, Rutten VPMG, Jansen CA (2020) Early life inoculation with
632 adult-derived microbiota accelerates maturation of intestinal microbiota and
633 enhances NK cell activation in broiler chickens. Front Vet Sci, 7, 584561.
634 https://doi.org/10.3389/fvets.2020.584561
635 Perré Y, Wauters A-M, Richard-Yris M-A (2002) Influence of mothering on emotional
636 and social reactivity of domestic pullets. Appl Anim Behav Sci, 75, 133-146.
637 https://doi.org/10.1016/S0168-1591(01)00189-7
638 Richard-Yris MA, Leboucher G (1987) Responses to successive test of induction of
639 maternal behaviour in hens. Behav Processes, 15, 17-26.
640 https://doi.org/10.1016/0376-6357(87)90030-1

641 Shimmura T, Kamimura E, Azuma T, Kansaku N, Uetake K, Tanaka T (2010) Effect of
642 broody hens on behaviour of chicks. Appl Anim Behav, 126, 125-133.
643 https://doi.org/10.1016/j.applanim.2010.06.011
644 Souza da Silva C, Molenaar R, Giersberg MF, Rodenburg TB, van Riel JW, De Baere
645 K, Van Dosselaer I, Kemp B, van den Brand H, de Jong IC (2021) Day-old chicken
646 quality and performance of broiler chickens from 3 different hatching systems.
647 Poult Sci, 100, 100953. https://doi.org/10.1016/j.psj.2020.12.050
648 Tona K, Bamelis F, De Ketelaere B, Bruggeman V, Moraes VM, Buyse J, Onagbesan
649 O, Decuypere E (2003) Effects of egg storage time on spread of hatch, chick
650 quality, and chick juvenile growth. Poult Sci, 82, 736-741.
651 https://doi.org/10.1093/ps/82.5.736
652 van de Ven LJ, van Wagenberg AV, Groot Koerkamp PW, Kemp B, van den Brand H
653 (2009) Effects of a combined hatching and brooding system on hatchability, chick
654 weight, and mortality in broilers. Poult Sci, 88, 2273-2279.
655 https://doi.org/10.3382/ps.2009-00112
656 van der Wagt I, de Jong IC, Mitchell MA, Molenaar R, van den Brand H (2020) A review
657 on yolk sac utilization in poultry. Poult Sci, 99, 2162-2175.
658 https://doi.org/10.1016/j.psj.2019.11.041
659 Volf J, Polansky O, Varmuzova K, Gerzova L, Sekelova Z, Faldynova M, Babak V,
660 Medvecky M, Smith AL, Kaspers B, Velge P, Rychlik I (2016) Transient and
661 prolonged response of chicken cecum mucosa to colonization with different gut
662 microbiota. PLoS One, 11, e0163932.
663 https://doi.org/10.1371/journal.pone.0163932